Human Genomics

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# Two novel mutations in *TBC1D32* add complexity to the oro-facial-digital syndrome

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## Abstract

**Background** Ciliopathies are characterized by the dysfunction of cilia, being inherited retinal dystrophies (IRDs) included in sensory ciliopathies. Besides, oro-facial-digital syndrome (OFD) is caused by mutations in ciliary genes, leading to dysmorphic features. Mutations in *TBC1D32* were associated to retinal dystrophy and OFD, defining this form as OFD-IX.

**Results** A clinical exome analysis performed on a patient presenting with OFD-IX and sensorineural hearing loss (SNHL) identified two variants in *TBC1D32*, one of which affects splicing, with its impact validated using a minigene assay.

**Conclusions** These results suggest that SNHL may represent a new clinical feature associated with this gene.

**Keywords** *TBC1D32*, Retinitis pigmentosa, Hearing loss, Minigene assay, Expanding clinical spectrum

## Background

Ciliopathies are a wide group of diseases characterized by the dysfunction of the cilia. Inherited retinal dystrophies (IRDs) are considered sensory ciliopathies as many IRD-causing genes are also in charge of cilium formation and its regulation in length [1]. They are characterized by the degeneration of the retinal pigmentary epithelium (RPE) or photoreceptors cells [1]. This eventually cause vision loss, usually being progressive. IRDs can also be manifested as a part of a syndrome, for which more than 200 genes have described to be causative [2].

*TBC1D32* was reported as a ciliary gene causing Oro-Facial-Digital syndrome type IX (OFD IX) [3]. This syndrome is clinically associated with facial cleft, microphthalmia/anophthalmia, coloboma, polydactyly and retinopathies [3]. In 2020, Hietamäki et al. were the first to identify *TBC1D32* variants in two siblings with OFD type IX and one also with RP (ophthalmologic data was only available for one sibling) [4]. Nevertheless, given the numerous tissues in where ciliary genes play a role,

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the clinical data around ciliopathies turns out to be very complex, such as clinical features for OFD type IX.

In the present work, we performed a clinical exome analysis in a patient who displayed a possible ciliopathy with other allegedly unrelated features, including IRD and hearing loss symptoms. Two compound heterozygous likely pathogenic variants were identified in *TBC1D32* that were not previously reported (c.2073del and c.769 + 5G > A). For c.769 + 5G > A, splicing functional studies have been carried out in order to confirm its pathogenicity. Moreover, this is the first study in which variants in *TBC1D32* have been identified in a patient displaying sensorineural hearing loss (SNHL). Since cilia are presented in inner ear, our results aim to suggest an expansion of the clinical spectrum of this *TBC1D32*-associated human ciliopathy with the inclusion of SNHL.

## Materials and methods

### Clinical evaluation

The patient underwent physical exploration, audiograms and several ophtalmological tests (see supplemental Materials and Methods). All the clinical studies were performed in the Hospital La Fe of Valencia, Spain. Moreover, the familiar and physiological background of the pregnancy was evaluated.

### Genetic and functional analysis

Several syndromes were discarded after analysing the responsible genes (Roiffman, Opitz-GBBB, Hypochondroplasia and Short Stature) through different techniques such as Sanger sequencing, genomic array and MLPA (see supplemental Materials and Methods).

A clinical exome sequencing (CES) was performed. The ClearSeq Inherited Disease Panel (Agilent Technologies) was used, which includes 3204 genes many of which were neurodevelopmental disorders genes. Particularly, a virtual gene panel containing 207 genes associated to retinal dystrophies were analysed. Afterwards, an updated version of CES including 5227 genes (Custom Constitutional Panel 17 MB; Agilent Technologies) was sequenced in the patient. Variant databases and in silico analysis, including splicing predictors, were assessed (see supplemental Materials and Methods).

CES was also performed on the DNA of available relatives to validate candidate variants and assess segregation patterns.

A minigene assay using the pSPL3 plasmid was conducted as previously reported [5]. After cloning and posterior transfection of the plasmid including the target region, cDNA from the extracted RNA was studied by Sanger sequencing (see supplemental Materials and Methods).

## Results

### Clinical findings

A 10-month-old boy was referred for evaluation for skeletal dysplasia suspection. The clinical examination showed several features that seemed to be highly suspected of genetic origin (Fig. 1) (see supplemental Materials and Methods). At the age of 12 years old, he was diagnosed with retinal dystrophy (Fig. 1A). Complementary exploration by MRI also showed absent of septum pellucidum with mid-line fusion of both fornices and an abnormal sulcation of both hippocampi (Fig. 1B). A complete clinical description is described in Table S1. Furthermore, a moderate bilateral sensorineural hearing loss at medium and high frequencies was observed (Fig. 1C), among several auditory malformations (see supplemental Results section).

### Molecular findings

After sequencing the updated clinical exome in the patient, we identified two likely pathogenic variants in heterozygous state, c.2073del - p.(Gly693Aspfs*15) in exon 18 and c.769 + 5G > A in intron six, both in *TBC1D32* (Figure S1). No hearing loss-causing mutations were identified. *TBC1D32* variants were validated in the parents through clinical exome sequencing (NGS) to perform the segregation analysis, confirming that the variants were in trans (Fig. 2A).

We performed functional validation for the intronic variant c.769 + 5G > A in *TBC1D32* based on these in silico predictions (see supplemental Results section). The outcomes predicted a skipping of exon six of *TBC1D32*. These predictions were validated by minigene assays (Fig. 2B) and the resulted products were sequenced by Sanger to confirm the exon skipping effect (Fig. 2C). Additionally, the creation of a new stop codon in exon seven (p.(Asp231Leufs*21)) was predicted by ORF Finder in the mutated sequence.

## Discussion

*TBC1D32* was reported as a ciliary gene in 2012 [6], which encoded TBC1D32 protein. This protein regulates the structure of the primary cilium in neural tube in Zebrafish *tbc1d32* morphants and in a murine *TBC1D32* knockout model [7]. Moreover, a recent study in a *Xenopus* model showed that *tbc1d32* knockdown altered the differentiation of RPE and photoreceptors, as well as caused defects in the ciliogenesis [8]. Additionally, analyses in fibroblasts cells from a patient harbouring mutations in *TBC1D32* confirmed that the protein is located in the centrosome of primary cilium and the mutations provoke cilium elongation defects [8].

*TBC1D32* is associated to OFD type IX syndrome [3], although it is not yet associated to any pathology in OMIM. Later, other clinical signs have been appearing

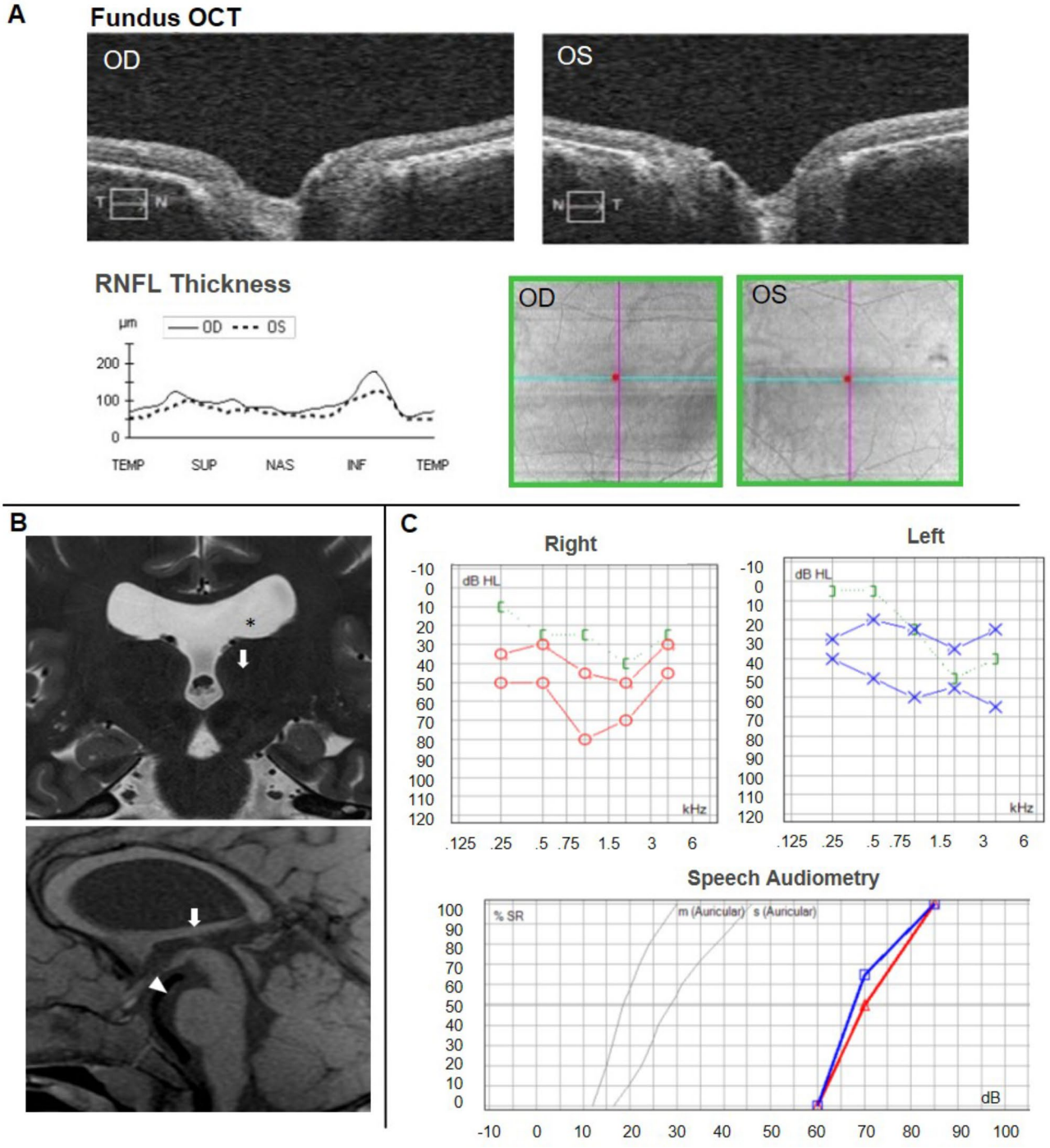


**Fig. 1** Clinical findings in index patient. (**A**) OCT of both eyes. (**B**) Brain MRI. The upper section shows coronal T2 weighted MRI with complete absence of the septum pellucidum (*) and mid-line fusion of both fornices (arrow). Abnormal sulcation of both hippocampi is also noted. The lower section shows sagittal T1 weighted of the mid-line brain: abnormal thickenned fornix (arrow) and ectopic neurohypophisis (arrowhead). Hypoplastic sella turcica. (**C**) Audiograms of both ears confirm a moderate bilateral SNHL in medium and high frequencies. The speech audiometry shows a 50% of understanding achieved at 50 decibels and 100% at 80 decibels bilaterally

in patients harbouring mutations in *TBC1D32*, including cases with isolated inherited retinal dystrophy [3, 8] (table S1). An association between IRD and OFD-IX is now clearly established, and research conducted by Bocquet et al.. has provided compelling evidence of the connection between the *TBC1D32* gene and ophthalmological involvement or IRD [8]. This is why, today, the *TBC1D32* gene is included in RetNet (https://web.sph.ut

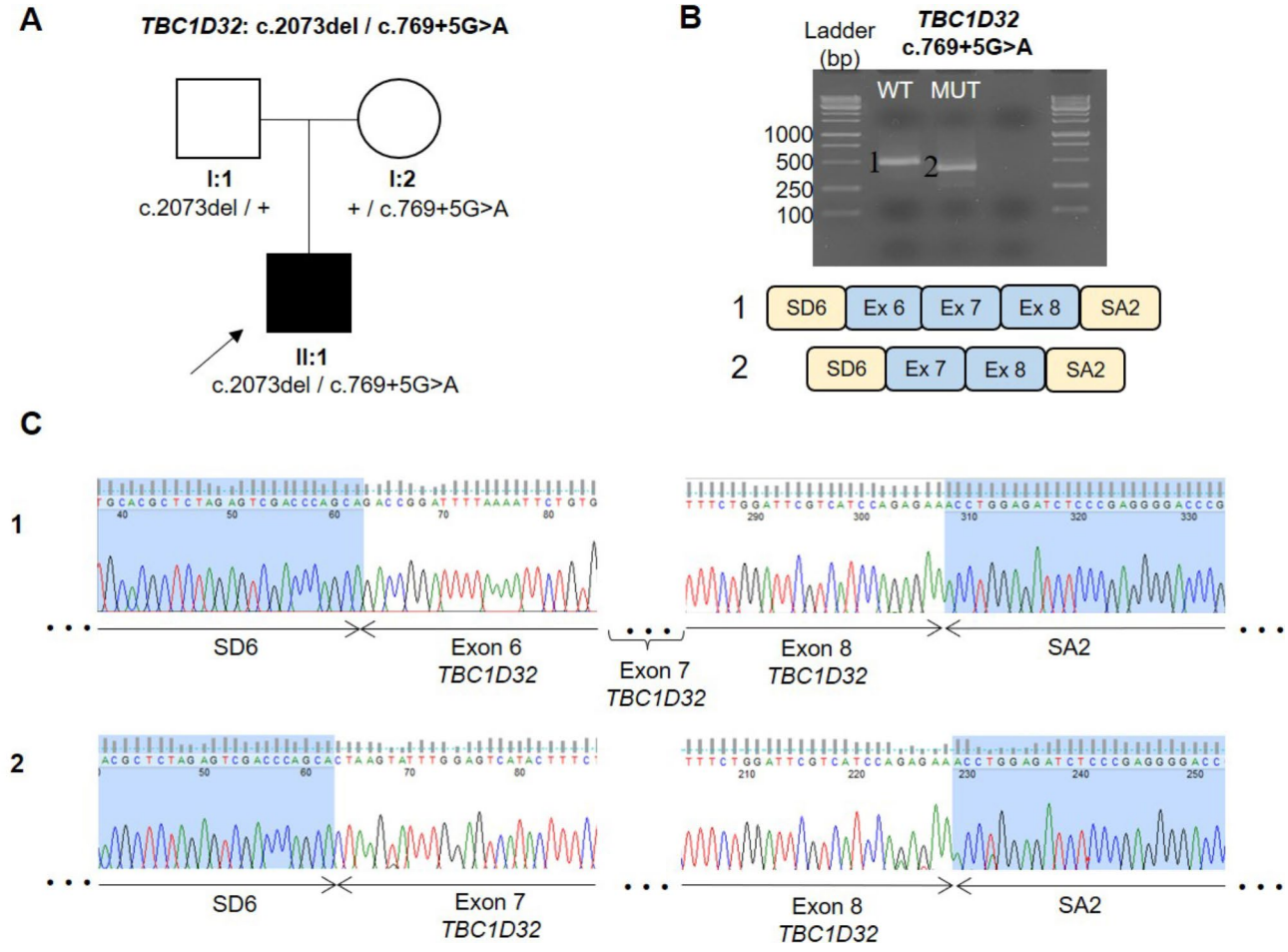


**Fig. 2** Segregation analysis of identified variants and minigene assay performed for variant *TBC1D32* (NM_152730): c.769 + 5G > A. (**A**) Segregation analysis performed in the parents confirming their co-segregation with the disease. (**B**) Amplification of the target region in the cDNA both wild-type and mutant as a result of the functional assay by minigene of variant c.769 + 5G > A. SD6 and SA2 represent the constitutive exons of the plasmid pSPL3. Wild-type and mutant contexts are depicted by bands 1 and 2, respectively, where band 2 depicts the exon 6 skipping; (**C**) cDNA sequence by Sanger of bands 1 and 2 observed in the gel of section B

h.edu/RetNet/home.htm). Our patient presents a clinical picture compatible with OFD IX in association with IRD and, moreover, audiograms showed moderate bilateral SNHL.

To date, no patient with mutations in *TBC1D32*, who manifested SNHL, had been described. We cannot completely rule out the possibility that this clinical sign is caused by genetic alterations that escape detection by technologies used, involve a gene not yet associated with hearing impairment, or even associated to non-genetic causes. However, as no variant in a SNHL-causing gene was identified, and considering the multitude of ciliopathies associated with defects in inner ear cilia [9], it is tempting to speculate that alterations in *TBC1D32* may also involve SNHL. Particularly, mutations in *TBC1D24*, a TBC1 domain family gene, were identified in three family displaying non-syndromic recessive hearing loss [10]. Additionally, a ciliopathy mouse model, knockout for *Bromi* gene (homologue of *TBC1D32*), presented cochlear shortening with a decrease in hair cells that also were developed prematurely, and an impaired sonic hedgehog pathway signalling as a consequence of defects in the primary cilium ciliogenesis [11], among others. Similarly, Bocquet et al., detected expression of *tbc1d32* in the otic vesicle of their *Xenopus* model and hair bundles alterations [8]. Thus, it is plausible to correlate OFD IX with hearing loss and this study could potentially broaden the phenotype associated with *TBC1D32*. These findings raise the possibility that OFD IX may be associated with hearing loss, potentially broadening the phenotype linked to *TBC1D32*.

Our findings raise the total number of pathogenic variants described in *TBC1D32* to 19 so far (five of them in non-coding regions) (Figure S1). Our study is the first in which minigene assay has been done to confirm the effect of a variant located in a non-canonical splice position. This highlights the necessity of carrying out functional

assays that confirm the effect in splicing of variants that do not alter any canonical splice site.

The lack of information about the domains and TBC1D32 protein's structure makes difficult to predict a genotype-phenotype correlation. To date, the TBC domain, located at C-terminal region, is the only defined in its sequence. Differences in the severity of the protein disruption and the residual protein function have been proposed as a possible explanation for the wide clinical variability observed in patients harbouring alterations in this gene [8]. In addition, hearing loss may have been underdiagnosed in previously reported cases, particularly if mild or not systematically assessed. The limited number of reported patients and the broad phenotypic spectrum observed highlight the need for more detailed clinical characterization and functional studies in future cases.

## Conclusions

In conclusion, the study of the *TBC1D32* gene, combined with functional studies and a thorough clinical evaluation, has allowed us to open the possibility of including SNHL as a new clinical sign. The wide clinical spectrum of *TBC1D32* variants highlights the complexity of ciliopathies and the importance of comprehensive genetic and clinical evaluations. This research contributes to refine the genetic diagnosis and broaden our understanding of *TBC1D32*-related ciliopathies.

### Abbreviations

| | |
|---|---|
| IRD | Inherited retinal dystrophies |
| RPE | Retinal pigment epithelium |
| OFD | Oro-facial-digital syndrome |
| SNHL | Sensorineural hearing loss |
| CES | Clinical exome sequencing |
| NGS | Next-generation sequencing |
| MLPA | Multiplex ligation-dependent probe amplification |
| MRI | Magnetic resonance imaging |
| ORF | Open reading frame |
| OMIM | Online mendelian inheritance in man |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40246-025-00759-0.

Supplementary Material 1

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### Author contributions

Conceptualization: GGG and JMM; Methodology: BGB, EA, PBM, MA, RLLS, ICAP; Formal analysis: BGB, EA; Investigation: BGB; Resources: BGB, EA, PBM, MA, RLIS and ICAP; Writing – original draft: BGB; Writing – review and editing: BGB, EA, PBM, MA, RLLS, ICAP, JMM and GGG; Supervision: JMM and GGG.

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### Data availability

The datasets generated from the analysis during this study is available from the corresponding author when requested.

## Declarations

### Ethics approval and consent to participate

This study was approved by the Hospital La Fe Ethics Committee, in agreement with the Declaration of Helsinki. A clinical questionnaire and an informed consent were completed by the patient and relatives.

### Consent for publication

Consent for publication was provided by patient and relatives.

### Competing interests

The authors declare no competing interests.

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